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Antineoplasic activity of *Copaifera multijuga* oil and fractions against ascitic and solid Ehrlich tumor

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ABSTRACT

The aim of this study was to investigate the effect of chronic treatment with *C. multijuga* oil on Ehrlich tumor evolution. *C. multijuga* was fractionated in a KOH impregnated silica gel column chromatography to give three distinct fractions, i.e., hexanic, chloroformic, and methanolic, mainly composed by hydrocarbon sesquiterpenes, oxygenated sesquiterpenes and acidic diterpenes, respectively. Results demonstrated that the *C. multijuga* oil, the hexanic, and chloroformic fractions did not develop toxic effects. The oil, hexanic and chloroformic fractions (doses varying between 100 and 200 mg/kg) showed antineoplasic properties against Ehrlich ascitic tumor (EAT) and solid tumor during 10 consecutive days of treatment inhibiting ascitic tumor cell number, reverting medulla and blood cell counts to values similar to control group, and inhibiting the increase on several inflammatory mediators (total protein, PGE_2, nitric oxide, and TNF) on ascitic fluid. The treatment also inhibited the increase in paw volume on tumor-inoculated mice. In conclusion, *C. multijuga* as well as its fractions demonstrated antineoplasic effect even after oral administration confirming its use by traditional medicine.

1. Introduction

The oil obtained from the trunk of *Copaifera* species (Fabaceae) is widely used by Amazonian people and is known as "copaiba", "copaíva" or "pau-de-óleo". The oil has been extensively used in folk medicine as anti-inflammatory, antitumoral, antitetanus, antiblenorrhagea, as urinary antiseptic, to treat bronchitis, syphilis, skin diseases, ulcers, as well as for healing wounds (Paiva et al., 2004a). Anti-inflammatory (Basile et al., 1988), anti-ulcerogenic (Paiva et al., 2004b), antitumoral against Walker sarcoma (Ohsaki et al., 1994) and melanoma cell line (Lima et al., 2003), and others activities have already been described. Copaiba oil is well known for their terpenoidic composition, mainly hydrocarbon and oxygenated sesquiterpenes (oxides and alcohols are more representative) and acidic diterpenes. Depending on the specie, the proportion between these metabolites can vary considerably (Pinto et al., 2000). *Copaifera multijuga* is composed of a high amount of hydrocarbon and a minor amount of oxygenated sesquiterpenes, together contributing to almost 90% of the total oil composition, followed by a small amount of acidic diterpenes. The main constituent is *trans*-β-caryophyllene, which is found as one of the most abundant sesquiterpenes in copaiba oils and appears to be ubiquitous in angiosperms (Pinto et al., 2000).

The Ehrlich tumor was initially described as a spontaneous murine mammary adenocarcinoma. It is a rapidly growing carcinoma with very aggressive behavior and is able to grow in almost all mice strains (Chen and Watikins, 1970; Segura et al., 2000). It has been used as a transplantable tumor model to investigate the antineoplasic effects of several chemical compounds. After intraperitoneal inoculation of Ehrlich tumor cells, the ascitic volume and cells number increase drastically. This has been associated to an increase in peritoneal vascular permeability (Fastaia and Dumont, 1976).

In this paper we describe the antineoplasic activity of *Copaifera multijuga* Hayne oil and isolated fractions on Ehrlich tumor in the solid and ascitic forms.

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2. Materials and methods

2.1. Plant material

C. multijuga Hayne oil, directly exuded from the trunk of the tree, was collected at Reserva Ducke, Manaus, Amazonas, Brazil. A sample is deposited at Herbarium of INPA (Manaus, Amazonas, Brazil), under the number 82,426.

2.2. Preparation of C. multijuga oil fractions

The oil was submitted to an open silica gel column chromatography impregnated with a KOH methanolic solution, in order to modify the selectivity of the adsorbent. The column was eluted with hexane, chloroform and methanol, consecutively, to yield three fractions with increasing polarities (hexanic, chloroformic and methanolic fractions). This procedure was described in detail by Braga et al. (1998) and Pinto et al. (2000) for *C. cearensis*.

2.3. Animals

All experiments were performed with male Swiss mice (20–25 g) or Wistar rats (150–200 g) obtained from our own animal facility. Animals were maintained in a room with controlled temperature 22 ± 2 °C for 12 h light/dark cycle, with free access to food and water. Animals were killed in a chamber with saturated CO_2 atmosphere due to hemorrhage to peritoneal cavity. Animal care, research and animal sacrifice protocols were in accordance with the principles and guidelines adopted by the Brazilian College of Animal Experimentation (COBEA) and approved by the Biomedical Science Institute/UFRJ—Ethical Committee for Animal Research.

2.4. Treatment regimen

C. multijuga oil, hexanic, and chloroformic fractions were dissolved in sterile corn oil and administered by oral gavage at the doses of 100, 150, and 200 mg/kg, in a final volume of 0.1 ml per animal. The control group was composed by vehicle (PBS, phosphate buffer saline, containing the same amount of corn oil). Positive control group was composed by vincristine (0.5 mg/kg). Mice received *C. multijuga* oil, fractions, vehicle, and vincristine every 24 h after inoculation of Ehrlich ascitic tumor cells until the end of experiment.

2.5. Ehrlich ascitic and solid tumor

The Ehrlich ascitic tumor (EAT), derived from a spontaneous murine mammary adenocarcinoma, was maintained in the ascitic form by sequential passages in Swiss mice, by means of weekly i.p. transplantations of 5×10^5 tumor cells. To the experiments with solid tumor, 5×10^5 tumor cells were injected in a volume of 0.1 ml in the footpad of rats and the contra-lateral paw received vehicle (Kleeb et al., 1997). Every 24 h and until the 6th day, paw edema was measured by pletismography as described by Ferreira (1979). To the experiments with ascitic tumor, mice received i.p. inoculation of 5×10^5 tumor cells in 0.5 ml. At the 10th day after tumor implantation mice were sacrificed. Samples of blood, medulla lavage and ascitic fluid were collected to several measurements as described above.

2.6. Collection of medulla lavage

Bone marrow cells were obtained by flushing the femoral cavity with 1 ml of PBS. The total number of cells was counted in the Newbauer chamber. Differential counts were obtained on the basis of 500 cells per slide in cytocentrifuge smears stained with May-Grünwald and Giemsa dyes.

2.7. Total and differential cell counts

The ascitic fluid collected was centrifuged at 170 × *g* for 10 min at 4 °C. The supernatant was collected to prostaglandin E_2, TNF-α, protein and nitric oxide dosages. The cell pellet was resuspended in 0.5 ml of PBS. An aliquot from ascitic cell suspension was diluted 1:20 in Turk solution (20% acetic acid containing 0.5% Trypan Blue). The total number of cells was determined by counting in a Newbauer chamber. Differential cell counts were performed after cytocentrifugation of ascitic fluid and staining with May-Grünwald and Giemsa dyes.

2.8. Protein, PGE_2, and TNF quantification

The concentration of protein was determined by the BCA method (BCA™ Protein Assay Kit, Pierce). The concentration of PGE_2 was determined by using EIA commercial kits (Cayman Chemical Co., MI, USA), according to the method of Pradelles and Maclouf (1985). Briefly, dilutions of the supernatants were incubated with the conjugated eicosanoid–acetylcholinesterase and with the specific antiserum in 96-well plates pre-coated with anti-rabbit immunoglobulin G antibodies. After overnight incubation at 4 °C, the plates were washed and the enzyme substrate (Elmman's reagent) was added for 60–120 min at 25 °C. The optical density of the samples was determined at 412 nm on a microplate reader, and the concentration of PGE_2 was calculated from a standard curve. TNF activity in the ascitic fluid was determined by bioassay using L929 cells based on the method described by Flick and Gliford (1984).

2.9. Quantification of nitric oxide (NO) production

To evaluate NO production, nitrate concentration in the ascitic fluid was measured using the nitrate conversion protocol (Bartholomew, 1984) followed by the Griess reaction (Green et al., 1982). Briefly, equal volume of ascitic fluid and of Griess reagent (1% sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride, 10% phosphoric acid) were incubated for 10 min at room temperature. The absorbance was measured at 540 nm using a Softmax microplate reader, and the nitrite concentration was calculated using a standard curve of sodium nitrite.

2.10. Statistical analysis

All experimental groups for *in vivo* protocols were composed by six to eight animals. For *in vitro* assays each group was done in triplicate and each protocol was repeated at least four times. The results are presented as the mean ± S.D. Statistical significance between groups was performed by the application of analysis of variance ANOVA followed by Bonferroni's test. Significant levels were defined as the *p*-value being less than 0.05.

3. Results

3.1. Effect of C. multijuga oil and fractions on Ehrlich ascitic tumor volume

Intraperitoneal injection of EAT cells resulted in appearance of an ascitic liquid. Maximal volume was achieved at the 10th day, after which no significant increase on volume was observed. The increase on ascitic volume was accompanied by an enlargement on total cell count that reached its maximum level at the 10th day

(Fig. 1A). After this period an intense hemorrhage was observed and animals died between 12 and 14 days. In view of this fact, all experiments were performed at the 10th day. Daily treatment of animals with 100, 150, or 200 mg/kg of *C. multijuga* oil resulted in a significant inhibition of total ascitic volume collected at the 10th day (5.8 ± 2.1 ml in control group vs. 3.8 ± 1.1 ml, 2.2 ± 0.9 ml, 1.9 ± 0.8 ml in 100, 150, and 150 mg/kg *C. multijuga*-treated group, respectively). Similar results were obtained with hexanic and chloroformic fractions resulting in a significant reduction on total ascitic volume (Fig. 1B). Daily treatment of mice with methanolic fraction resulted in toxic effect achieving death of animals. In view of this fact this fraction was not used to further experiments.

3.2. Effect of *C. multijuga* oil and fractions on blood and medulla cell lavage

At the 10th day after inoculation of EAT cells into mice, a significant increase of 12.6-fold on total blood cell count was observed ($0.5 \pm 0.2 \times 10^7$ cells in control group vs. $6.3 \pm 2.1 \times 10^7$ cells in EAT-inoculated mice). This effect was accompanied by a proportional increase on total medulla cell count ($0.12 \pm 0.02 \times 10^7$ cells in control group vs. $1.3 \pm 0.02 \times 10^7$ cells in EAT-inoculated mice). Fig. 2 shows that vincristine (at 0.5 mg/kg) reduced total blood cell count in 59.5% ($6.3 \pm 2.1 \times 10^7$ cells in EAT-inoculated mice vs. $2.8 \pm 0.8 \times 10^7$ cells in vincristine-treated EAT inoculated mice) and total medulla cell count in 76.9% ($1.3 \pm 0.02 \times 10^7$ cells in EAT-inoculated mice vs. $0.3 \pm 0.2 \times 10^7$ cells in vincristine-treated EAT inoculated mice). Treatment of animals previously inoculated with

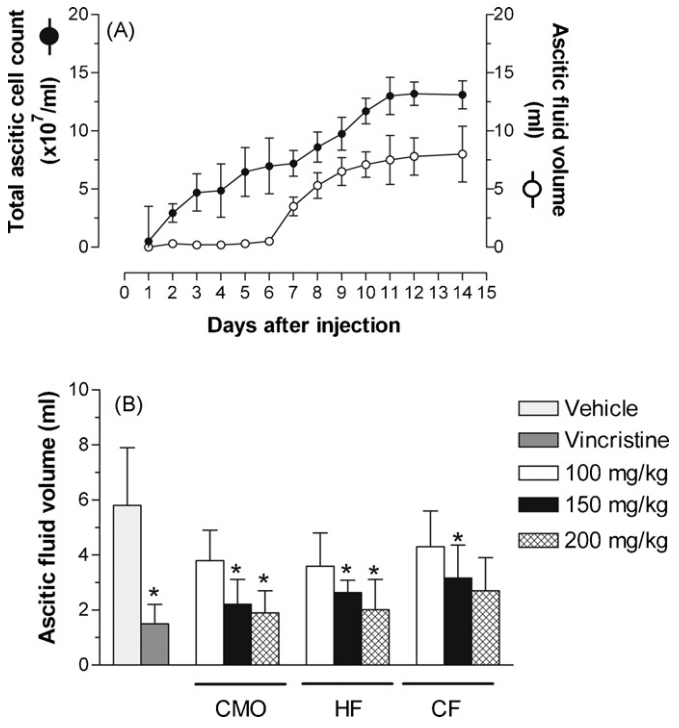

Fig. 1. Effect of *C. multijuga* oil, hexanic, and chloroformic fractions against Ehrlich ascitic tumor-bearing mice. Mice were i.p. inoculated with 5×10^5 Ehrlich ascitic tumor cells. In (A), every 24 h after inoculation a group of mice was sacrificed. Ascitic fluid was collected and volume and total cell count was performed as described on methods section; in (B), 24 h after EAT inoculation and during the subsequent days, mice were treated with vincristine (0.5 mg/kg), *C. multijuga* oil, hexanic, or chloroformic fraction (100, 150, and 200 mg/kg). On the 10th day the mice were sacrificed and ascitic fluid volume measured. Statistical significance was calculated by ANOVA followed by Bonferroni's test ($n = 6$–8). $^*p < 0.05$ when compared vincristine, *C. multijuga* oil, hexanic, or chloroformic fraction-treated mice with vehicle-treated group. CMO = *C. multijuga* oil; HF = hexanic fraction; CF = chloroformic fraction.

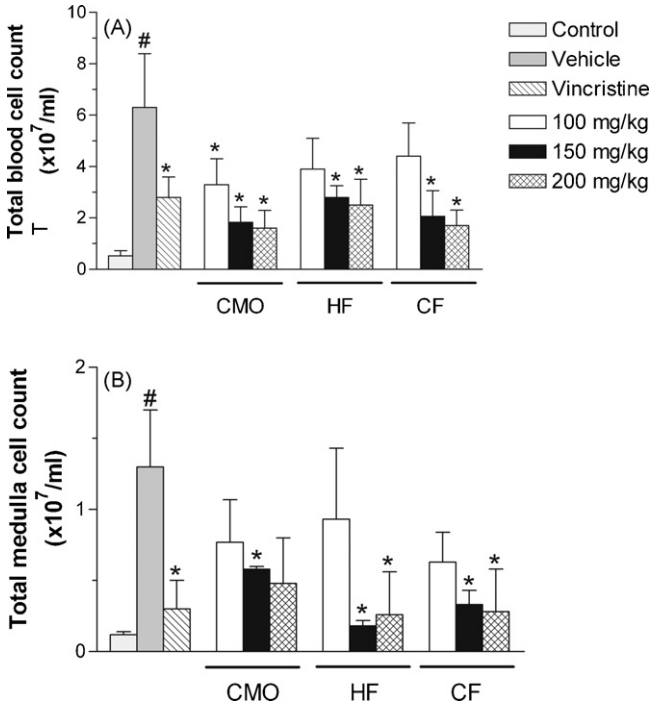

Fig. 2. Effect of *C. multijuga* oil, hexanic, and chloroformic fractions on total blood and medulla cell count. Mice were i.p. inoculated with 5×10^5 Ehrlich ascitic tumor cells. Every 24 h after inoculation mice were treated with vincristine (0.5 mg/kg), *C. multijuga* oil, hexanic, or chloroformic fraction (100, 150, or 200 mg/kg). On the 10th day mice were sacrificed. Ascitic fluid was collected and total cell count on blood (A) and medulla (B) were performed as described in Section 2. Statistical significance was calculated by ANOVA followed by Bonferroni's test ($n = 6$–8). $^\#p < 0.05$ when comparing vehicle-treated mice with control group and $^*p < 0.05$ when comparing vincristine, *C. multijuga* oil, hexanic, or chloroformic fraction-treated mice with vehicle-treated group. CMO = *C. multijuga* oil; HF = hexanic fraction; CF = chloroformic fraction.

EAT cells with crescent doses of *C. multijuga* oil, as well as hexanic and chloroformic fractions, reduced in a dose-dependent manner both total blood and medulla cell counts to values similar to vincristine-treated mice.

Fig. 3A–C shows that the pretreatment of mice with 150 mg/kg of *C. multijuga* oil or its fractions significantly inhibited the increase in monocytes, neutrophils, and eosinophils numbers on blood. In a similar manner, *C. multijuga* oil and its fractions also reduced the values of differential cell counts (monocytes and eosinophils) in medulla (Fig. 3D and E). However, neither *C. multijuga* oil nor its fractions altered eosinophils counts (Fig. 3F).

3.3. Effect of *C. multijuga* oil and isolated fractions on inflammatory mediators production

In order to evaluate inflammatory mediators after EAT inoculation, mice were sacrificed at the 10th day after EAT injection. Peritoneal ascitic fluid was collected and total protein, nitric oxide, PGE_2, and TNF-α were measured. In mice inoculated with EAT cells there was a drastic increase on these parameters when compared to non-inoculated animals. Treatment of mice with *C. multijuga* oil (100, 150, and 200 mg/kg) reduced significantly the total protein extravased (in 16.4, 25.8, and 30.8%), the production of nitric oxide (in 13.8, 33.3, and 42.4%), the PGE_2 production (in 8.2, 17.7, and 23.5%), and TNF-α liberation (in 15.6, 19, and 24.6%). Hexanic and chloroformic fractions also reduced these parameters. The inhibitory effect of the hexanic fraction was comparable to the *C. multijuga* oil but the chloroformic fraction was more prominent. The doses of 100, 150, and 150 mg/kg

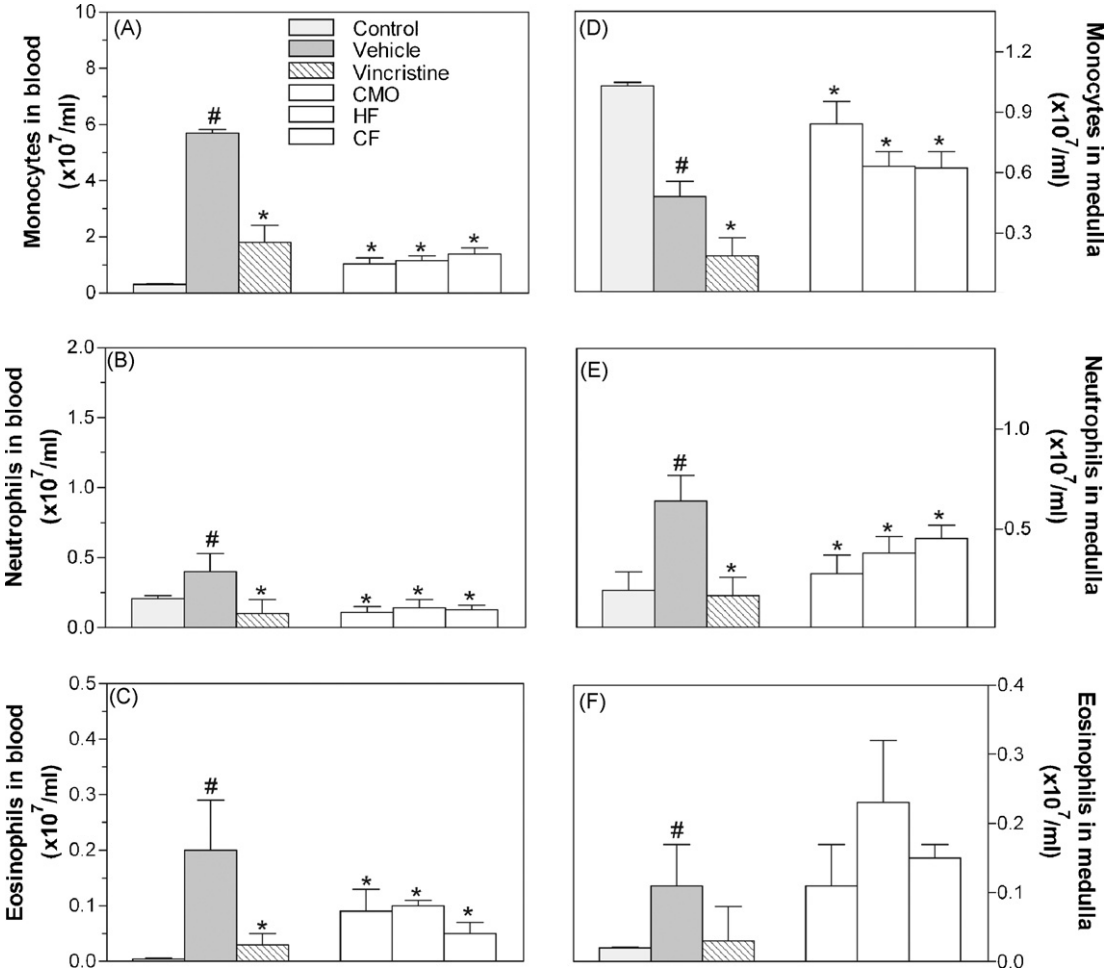

Fig. 3. Effect of *C. multijuga* oil, hexanic, and chloroformic fractions on differential blood and medulla cell count. Mice were i.p. inoculated with 5×10^5 Ehrlich ascitic tumor cells. Every 24 h after inoculation mice were treated with vincristine (0.5 mg/kg), *C. multijuga* oil, hexanic, or chloroformic fraction (150 mg/kg). At the 10th day mice were sacrificed. Differential (monocytes, neutrophils, and eosinophils) cell counts on blood (A–C) and on medulla (D–F) were performed as described in Section 2. Statistical significance was calculated by ANOVA followed by Bonferroni's test ($n = 6$–8). $^{\#}p < 0.05$ when comparing vehicle-treated mice with control group and $^{*}p < 0.05$ when comparing vincristine, *C. multijuga* oil, hexanic, or chloroformic fraction-treated mice with vehicle-treated group. CMO = *C. multijuga* oil; HF = hexanic fraction; CF = chloroformic fraction.

reduced total protein leakage by 28.4, 35.3, and 39.3%, nitric oxide production by 51.1, 72.8, and 76.1%, PGE_2 production in 28.2, 61.5, and 64.2%, and TNF-α liberation by 20.7, 30, and 32%, respectively. The antitumoral drug, vincristine (0.5 mg/kg) also significantly reduced production of inflammatory mediators (Table 1).

3.4. Effect of *C. multijuga* oil and fractions on solid Ehrlich tumor

In order to evaluate if *C. multijuga* oil and fractions demonstrate antitumoral effect against a solid tumor, the rats received intraplantar injection of 5×10^5 EAT cells. Daily measurement of rats paw edema showed an increase of tumor that reached a plateau at the

Table 1
Effect of *C. multijuga* oil, hexanic, and chloroformic fractions on inflammatory mediators produced on ascitic fluid after EAT cells inoculation

Treatment	mg/kg	Total protein (mg/ml)	NO (μM)	PGE_2 (pg/ml)	TNF-α (U/ml)
Vehicle	–	493.5 ± 33.8	76.9 ± 12.7	40.0 ± 0.6	792.3 ± 113.4
Vincristine	0.5	139.4 ± 21.8*	28.9 ± 9.9*	18.3 ± 6.7*	361.8 ± 51.4*
C. multijuga oil	100	410.9 ± 41.5	66.3 ± 13.4	36.7 ± 9.9	668.9 ± 39.7*
	150	371.8 ± 44.6*	51.3 ± 6.6*	32.9 ± 3.4*	641.8 ± 97.5*
	200	341.6 ± 33.7*	44.3 ± 9.8*	30.6 ± 6.8*	597.6 ± 44.7*
Hexanic fraction	100	385.4 ± 66.7	58.6 ± 15.4*	34.8 ± 9.4	649.5 ± 54.3*
	150	363.9 ± 26.7*	43.8 ± 8.7*	32.6 ± 11.2	621.6 ± 61.7*
	200	336.8 ± 26.8*	39.7 ± 8.7*	29.7 ± 8.7*	613.2 ± 62.4*
Chloroformic fraction	100	353.2 ± 29.3*	37.6 ± 16.3*	28.7 ± 6.9*	628.2 ± 54.3*
	150	319.4 ± 36.1*	20.9 ± 6.5*	15.4 ± 7.5*	553.9 ± 46.8*
	200	299.7 ± 26.8*	18.4 ± 9.8*	14.3 ± 6.6	539.1 ± 48.7*

*Statistical significance was calculated by ANOVA followed by Bonferroni's test ($n = 6$–8). $^{*}p < 0.05$ when compared *C. multijuga* oil, hexanic, or chloroformic fraction-treated mice with vehicle-treated group.

Table 2
Effect of *C. multijuga* oil, hexanic, and chloroformic fractions on solid Ehrlich tumor

Group	mg/kg	Days after tumor inoculation					
		1	2	3	4	5	6
Vehicle	–	174.0 ± 40.9	198.5 ± 33.1	220.1 ± 22.0	225.9 ± 32.0	249.7 ± 21.4	264.8 ± 12.0*
Vincristine	0.5	98.7 ± 15.3*	88.6 ± 7.7*	97.9 ± 11.3*	103.5 ± 12.3*	115.7 ± 21.4*	135.7 ± 17.6*
CMO	100	171.8 ± 18.3	177.6 ± 21.4	138.3 ± 19.1*	143.9 ± 15.8*	132.3 ± 14.1*	128.7 ± 15.6*
	150	156.4 ± 34.0*	136.4 ± 21.0*	100.3 ± 15.0*	70.4 ± 10.0*	72.0 ± 11.4*	71.0 ± 23.0*
	200	144.8 ± 21.3*	129.7 ± 19.8*	96.8 ± 10.3*	71.8 ± 9.8*	68.6 ± 12.1*	66.9 ± 6.9*
Hexanic	100	176.7 ± 18.3	143.5 ± 19.7*	129.2 ± 21.3*	113.8 ± 15.2*	99.5 ± 10.3*	95.4 ± 10.1*
frac-	150	175.9 ± 45.0	129.0 ± 25.0*	110.9 ± 15.0*	87.1 ± 11.0*	80.4 ± 12.0*	81.4 ± 12.0*
tion	200	165.3 ± 26.9	101.4 ± 13.3	107.6 ± 9.7*	65.4 ± 19.7*	69.8 ± 9.9*	68.5 ± 9.1*
Chloroformic	100	169.4 ± 19.9	119.7 ± 12.6*	103.8 ± 13.4*	81.3 ± 13.8*	67.8 ± 11.3*	61.3 ± 7.6*
frac-	150	166.8 ± 25.0	106.9 ± 13.8*	97.4 ± 10.6*	71.4 ± 6.8*	66.8 ± 5.9*	55.8 ± 6.9*
tion	200	155.4 ± 22.8	99.7 ± 6.9*	88.4 ± 10.7*	66.5 ± 8.5	55.9 ± 9.8*	44.8 ± 7.1

*Statistical significance was calculated by ANOVA followed by Bonferroni's test ($n = 6–8$). *$p < 0.05$ when compared *C. multijuga* oil, vincristine, hexanic, or chloroformic fraction-treated rats with vehicle-treated group.

6th day after inoculation (Table 2). After this time paw edema did not increase and a necrotizing tissue appeared (data not shown). Treatment of rats with *C. multijuga* oil or fractions at the doses of 100, 150, or 200 mg/kg for the same period significantly reduced the edema formation. There was no difference between treated groups. When treatment was prolonged over 6 days, animals did not develop the necrotizing tissue (data not shown). Administration of vincristine (0.5 mg/kg) also significantly reduced the paw edema formed.

4. Discussion

Copaifera multijuga Hayne oil and its fractions (hexanic and chloroformic) demonstrated significant inhibitory effect when administered by oral gavage on Ehrlich tumor growth in Ehrlich tumor-bearing mice.

C. multijuga oil was submitted to a KOH impregnated silica gel column chromatography as previously used in *C. cearensis* by Braga et al. (1998). Elution with hexane, chloroform and methanol gave three distinct fractions. By GC-MS, the most apolar fraction (obtained with hexane) gave hydrocarbon sesquiterpenes in 83% (w/w) of the total oil composition; with chloroform, gave oxygenated sesquiterpenes (4%, w/w) and with methanol, the acidic diterpenes were obtained as potassium salts and neutralized with aqueous HCl to give the free acids (9.0%, w/w).

Copalic acid, the biomarker of copaiba oils, is the main diterpene in *C. multijuga* (8.5%) as observed by gas chromatography after methylation with diazomethane/ethyl ether solution. Other major components are α-humulene, α-bergamotene and α-copaene in 8.6, 6.4 and 4.2%, respectively, besides minor compounds as β-bisabolene, δ-cadinene, α-elemene and δ-cadinol. Detailed composition of *C. multijuga* oil has already been described by Sant'Anna et al. (2007).

The pretreatment of Ehrlich tumor-bearing mice with *C. multijuga* oil, hexanic, or chloroformic fractions during 10 days after tumor inoculation resulted in a significant reduction on ascitic volume, total and differential cells count on blood and medulla indicating that *C. multijuga* oil, as well as its fractions, have powerful activity against its invasive tumor.

Ehrlich tumor is a rapidly growing carcinoma with very aggressive behavior (Segura et al., 2000). This seems that the controlling of Ehrlich tumor is more related to innate immunity, specially the inflammatory response. The neutrophilic inflammatory response is essential to Ehrlich tumor controlling. However, the high influx of these cells promotes tumor development (Bergamini-Santos et al., 2004). This effect is probably related with the angiogenesis and growing factors induced by inflammation that are necessary for tumor development. The Ehrlich ascitic tumor implantation induces *per se* a local inflammatory reaction, with increasing vascular permeability that results in an intense edema formation, cellular migration and progressive ascitic fluid formation (Fecchio et al., 1999). The ascitic fluid is essential for tumor growing since it constitutes the direct nutritional source for tumor cells (Gupta et al., 2004). One possible explanation for the reduction on ascitic fluid accumulated on peritoneal cavity of mice after treatment with *C. multijuga* oil and its fraction may be its well-related anti-inflammatory activity. There are several reports showing that different species of *Copaifera* develops anti-inflammatory effect (Basile et al., 1988; Paiva et al., 2004b). However, there are few reports demonstrating a significant effect of *C. multijuga* against tumor-inoculated animals. Ohsaki et al. (1994) reported antitumoral effect against Walker sarcoma cells. Our group demonstrated that *C. multijuga* oil administered by oral gavage reduced melanoma evolution on C57 black/10 mice previously inoculated with B16/F10 cell line (Lima et al., 2003).

Sesquiterpenes with the same skeleton found in *C. multijuga* have already been studied for their antitumoral activity. Wang et al. (2002) isolated five β-caryophyllene-derived sesquiterpene as alcohols, ketones and epoxides from the gorgonian coral *Subergorgia suberosa*, which showed significant cytotoxicity (Wang et al., 2002). Legault et al. (2003) described the antitumoral activity of balsam fir oil (*Abies balsamea* essential oil) against solid tumor cell lines. The cytotoxicity of the oil was associated to α-humulene and γ-caryophyllene after testing pure compounds. The essential oil of *Cordia verbenaceae* was found to be a powerful topical anti-inflammatory, which led to the development of the first approved phytomedicine completely developed in Brazil. After a detailed study of the oil composition, α-humulene and *trans*-β-caryophyllene were considered to be responsible for this activity (Tao et al., 2006). Also, α-bisabolol needs to be cited as the most famous bisabolane sesquiterpene known. It is considered the main contributing compound for the anti-inflammatory effect of chamomile (*Matricaria chamomilla*). Cavalieri et al. (2004) showed a strong time- and dose-dependence cytotoxic effect of α-bisabolol on human and rat glioma cells. The antineoplasic effect of *C. multijuga* oil demonstrated in this work are in accordance with others works which report the presence in the oil of many sesquiterpenes previously cited such as β-caryophyllene, bisabolene, and γ-caryophyllene.

In spite of doses used from copaiba oil, the fact that the *C. multijuga* oil and its fractions are not pure drugs and are not synthetic must be taken into account. They have different composition and different concentration of several constituents. Another important observation is that they were all administered orally. Influence of pH from stomach and lipossolubility may interfere with their absorption by gastrointestinal tract limiting the amount that reaches the blood and the tissues. Even so, with all these additional factors, an important antineoplasic effect was observed.

Our results demonstrated that *C. multijuga* oil and its fractions develop antineoplasic activity on Ehrlich ascitic and solid tumor. The mechanism by which the effect occurs must be fully investigated. This work intends to turn possible the design of less expensive therapies with minor adverse effects in treating tumoral processes, reinforcing the importance of copaiba oil as a phytomedicine.

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